matter. Applicants respectfully request the entry of these amendments, leaving claims 21-23, 25-27, and 39-42 pending in this application.

Rejection of claim 33 under 35 U.S.C. § 102(e)

The Office rejects claim 33 as allegedly anticipated by Markussen et al. (US Patent No. 4,916,212). (Office Action at pages 2-3.) That rejection is now moot as claim 33 is cancelled.

Rejection of claims 21 and 33-36 under 35 U.S.C. § 103(a)

The Office rejects these claims as allegedly obvious over Markussen et al. ("Markussen") in view of Goeddel et al. (EPO 055,945; "Goeddel"), Grau (US Patent No. 4,801,684; "Grau '684"), and Grau (US Patent No. 4,639,332; "Grau '332"). (Office Action at pages 3-6.) Applicants respectfully traverse this rejection, but note that only claim 21 is currently pending.

A *prima facie* case of obviousness must meet several essential criteria. First, the prior art references must teach or suggest all of the claim limitations. M.P.E.P. § 2142. In addition, there must be both a suggestion or motivation to modify the references or to combine their teachings and a reasonable expectation of success in performing the combination. *Id.* The motivation to combine the references and the reasonable expectation of success must both be found in the references themselves or in the knowledge generally available to one of ordinary skill in the art, and not in the applicant's disclosure. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991); M.P.E.P. § 2142.

The Office relies on Markussen for a teaching of mono-Arg-insulin, Goeddel for fusion proteins, and the two Grau patents for discussions of cleavages with trypsin and carboxypeptidase B. Applicants submit, however, that there insufficient motivation to

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combine Markussen with Goeddel and the two Grau references and no reasonable expectation of success in performing the combination. Thus, Applicants submit that the Office has not established a *prima facie* case of obviousness.

As the Office admits, Markussen does not describe the trypsin cleavage of step (d) of claim 21. (Office Action at page 4.) Instead, Markussen's "*in vitro* conversion" examples relate only to a process involving L-threonine esters. (*See* Markussen at col. 5, lines 3-11, and at Examples 14-18.) Markussen's disclosure of the L-threonine ester conversion process, in fact, suggests that one of ordinary skill in the art would not have expected trypsin to properly cleave at the C-terminus of the Arg residue in the mini-proinsulin compound that Applicants claim, according to Applicants' method of claim 21. Indeed, why would one use the L-threonine ester approach if the more direct trypsin digestion method was applicable?

Contrary to the Office's contentions, none of the other three references relieve this deficiency in Markussen. Thus, this combination of references, taken in light of the prior art, fails to show a reasonable expectation of success in performing the claimed trypsin cleavage of step (d) of claim 21. For example, Grau '684 describes trypsin and carboxypeptidase B cleavage of a different compound from that claimed here, and provides no assurance and no reasonable expectation of success that the same results would be obtained with the present, claimed mini-proinsulin compound. Grau '684 describes a ***natural porcine proinsulin*** isolated from the pancreas while the instant invention describes a mini-proinsulin of the formula B(1 - 30) – Arg - A(1 - 21).

Since it is known that trypsin cleaves at the carboxy terminus of the basic amino acids arginine and lysine, in porcine proinsulin, trypsin can cleave theoretically at 6

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different sites in the molecule, i.e., 2 sites in the B-chain, 3 sites in the C-chain and 1 site between C- and A- chain. Because the cutting rates (kinetics) of the possible cuts depend on the amino acid environment at each site, the actual cutting rates at the six different sites are different. In order for Grau '684 to achieve their invention, the cutting rates at Arg-(B22) and Lys-(B29) must be relatively low compared to the rate of the cutting site at Arg-(C35) since the process described by Grau '684 yields as the main product insulin with an intact B-chain.

Cutting off the mini-proinsulin from the fusion protein in the instant occurs at ***a different site*** from the splice site in the porcine insulin described by Grau '684. Applicants assert that the cutting rate of trypsin at this new site cannot be predicted in view of Grau '684. (*See* Applicants remarks filed June 6, 1997, at pages 12-13 for a thorough discussion of Grau '684.)

Similarly, Goeddel relates to different insulin precursors and does not suggest that pro-insulin with a C-chain consisting of only one amino acid can be properly cleaved proteolytically, as Applicants claim. (Goeddel at page 8, lines 8-31, and at page 34, lines 25-34.)

The Office relies on Grau '332 for a teaching that trypsin cleavage can form mono-Arg-insulin. (Office Action at page 4.) But, as the Board of Patent Appeals and Interferences pointed out in its Decision on Appeal, Grau '332 discloses a large number of possible precursor compounds. (Decision on Appeal at pages 7-8.) Further, its two examples demonstrate cleavages of preproinsulins with C-chains containing two arginines at positions 31 and 32 rather than a single arginine. (Grau '332 at Examples 1-2.) This presents a different cleavage environment for the trypsin enzyme. Thus,

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Grau '332 does not provide any specific teaching suggesting that the L-threonine ester conversion method of Markussen could successfully be substituted with a trypsin cleavage method as Applicants claim.

Finally, other references in the art such as Thim et al. ("Secretion and processing of insulin precursors in yeast," *Proc. Natl. Acad. Sci. USA*, 83: 6766-6770 (1986)) teach away from Applicant's method. Thim et al. points out that the efficacy of trypsin cleavage depends strongly on the structure of the C-peptide and the nature of the accessibility of the cleavage sites between the A- and B-peptides that results from changes to the C-peptide. (*See* Thim et al. at p. 6769, col. 2, particularly at the last incomplete paragraph.) Thim et al., at the last incomplete paragraph of page 6769, first two sentences, also informs the skilled artisan that trypsin would not cleave a mini-proinsulin with only an Arg-Arg or Lys-Arg bridge between the B and A chains. Thus, when the C-peptide is dramatically reduced in size, Thim et al. demonstrates that improper trypsin cleavage may result. (*See* Applicants' remarks at page 11 filed November 6, 1995, for further discussion of Thim et al. and a copy of the reference) Further, it is known in the art that Lys (B29), which is nearly adjacent to Arg (B31) in the claimed compound, is poorly accessible to trypsin. (*See, e.g.,* Kemmler et al., *J. Biol. Chem.* 246(22): 8786-91 (1971) at page 8786, col. 2, copy enclosed as Exhibit A.)

In summary, because there is insufficient motivation to combine these references and no reasonable expectation of success in performing the combination to obtain Applicants' claim 21, Applicants request the withdrawal of this rejection.

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Rejection of claims 25 and 37-38 under 35 U.S.C. § 103(a)

The Office rejects these claims as allegedly obvious over Markussen in view of Goeddel, Grau '684, and Grau '332, and further in view of Mai et al. (US Patent No. 5,087,564; "Mai"). (Office Action at pages 6-7.) Applicants respectfully traverse this rejection, but note that only claim 25 is currently pending.

Claim 25 recites a particular bridging amino acid sequence MIEGR (Met-Ile-Glu-Gly-Arg). The Office admits that this bridging sequence is not taught in any of the cited art. (Office Action at page 6.) Nevertheless, the Office concludes that Mai's disclosure would lead the skilled artisan to use the claimed bridging sequence. (*Id.*)

Mai discloses cleavage sites in prothrombin, which are acted upon by the serine protease factor Xa. (See Mai at col. 9.) While Mai discloses a proposed substrate recognition determinant of factor Xa that is IEGR, the remainder of the reference never suggests using this sequence in a fusion peptide. Rather, the reference teaches use of a recA-EGR or recA-E sequence. There is no suggestion to add an IEGR sequence to a fusion protein, much less a MIEGR sequence as in Applicants' method.

Clearly, the prior art references do not reasonably provide the ordinarily skilled artisan with a vision of the claimed bridging sequence for use in the claimed fusion protein. Thus, one skilled in the art would not be led to Applicants' invention based upon the teachings of Mai.

Moreover, Applicants have discussed the teachings of Markussen, Goeddel, and the two Grau patents in the preceding section, noting that these references in combination fail to provide a reasonable expectation of success in carrying out the trypsin cleavage as claimed in claim 21. Because claim 25 also includes the trypsin

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cleavage step, Applicants submit that those four references in combination do not render claim 25 obvious. Nor does Mai add any disclosure that would compensate for the deficiencies of Markussen, Goeddel, and the Grau patents in regard to the trypsin cleavage step because it does not discuss trypsin cleavage of insulin precursors.

For these reasons, Applicants submit that the Office has failed to establish a *prima facie* case of obviousness and request the withdrawal of this rejection.

Rejection of claims 22-23 and 40-41 under 35 U.S.C. § 103(a)

The Office rejects these claims as allegedly obvious over Markussen in view of Goeddel, Grau '684, and Grau '332. (Office Action at pages 7-8.) Applicants respectfully traverse this rejection.

The Office applies the same combination of references here as in its rejection of claims 21 and 33-37 discussed previously. Claims 22-23 and 40-41 differ from claim 21, for example, in reciting cleavage of mini-proinsulin with carboxypeptidase B as well as with trypsin.

Applicants submit that the Office has failed to establish a *prima facie* case of obviousness against claims 22-23 and 40-41 for the same reasons it has failed to do so against claim 21. Because the arguments presented above with reference to the rejection of claim 21 apply equally to claims 22-23 and 40-41, Applicants refer the Office to that section rather than reiterate those arguments here. For the reasons explained in that section, this combination of references fails to provide a reasonable expectation of success in performing trypsin cleavage to obtain a mono-Arg-insulin as Applicants claim. Thus, Applicants request the withdrawal of this rejection.

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Rejection f claims 26-27 and 31-32 under 35 U.S.C. § 103(a)

The Office rejects these claims as allegedly obvious over Markussen in view of Goeddel, Grau '684, Grau '332, and Mai. (Office Action at pages 8-10.) Applicants respectfully traverse this rejection, but note that only claims 26-27 are currently pending as claim 31 is now cancelled and that claim 32 was cancelled in a previous amendment dated March 2, 1998.

Applicants note that this same combination of references was applied to claim 25, as discussed in a previous section. Because claims 26 and 27 also recite the MIEGR bridging member and a trypsin cleavage step, Applicants submit this combination of references fails to rise to the level of a *prima facie* case of obviousness here for the same reasons described in the preceding sections discussing claims 21 and 25. (See the preceding sections discussing the rejections of claims 21 and 25.)

Further, there is no reasonable expectation of success that the alleged teaching of Grau '684 of the simultaneous addition of trypsin and carboxypeptidase would work with the mini-proinsulin of the present invention having the claimed bridging member. Cutting off the rest of the bridging sequence (IEGR) - after removal of the fusion part by cyanogen bromide - is provided by trypsin, which cuts also at other sites in the mini-proinsulin part of the fusion protein (Arg-(B22), Lys-(B29) and Arg-(C1)).

Grau '684 describes a natural porcine proinsulin isolated from the pancreas while the instant invention describes a mini-proinsulin of the formula B(1-30)-Arg-A(1-21). The cutting off of the mini-proinsulin from the bridging sequence occurs at a site which has no equivalent in the porcine insulin described by Grau '684. In addition, Applicants

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assert that the cutting rate of trypsin at this new site could not have been predicted in view of Grau '684. (See the remarks in the preceding section.)

Thus, there is nothing in the Grau '684 reference or the other prior art references of record that would suggest to the skilled artisan that the B(1-30)-Arg-A(1-21) mini-proinsulin having the MIEGR bridging sequence claimed by Applicants could be processed with the enzyme combination used in the Grau '684 reference.

Thus, Applicants request the withdrawal of this rejection.

Rejection of claims 39 and 42 under 35 U.S.C. § 103(a)

The Office rejects these claims as allegedly obvious over Markussen in view of Grau '684 and Grau '332. (Office Action at pages 10-11.)

Applicants respectfully traverse this rejection and request its withdrawal for the same reasons as discussed previously in relation to the rejection of claim 21. Step (b) of both of these claims recites trypsin cleavage of mini-proinsulin similar to step (d) of claim 21. Applicants submit above that Markussen and the two Grau patents do not provide a reasonable expectation of success in performing this cleavage to yield the compound of the formula II as claimed for the reasons discussed above in relation to the rejection of claim 21. For those reasons, Applicants request the withdrawal of this rejection.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

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Please grant any extensions of time required to enter this response and charge any required fees not submitted herewith to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: June 5, 2003

By: _____
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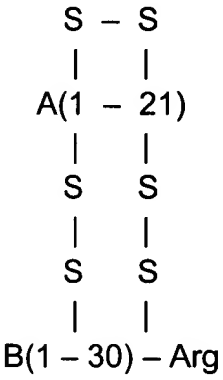
APPENDIX TO AMENDMENT OF JUNE 3, 2003

Version Showing Changes Marked-Up

In the Claims:

Please amend claim 42 as follows:

42. (Amended) A method for the preparation of a mono-Arg-insulin compound of the formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S-bridges are positioned as in insulin, which comprises:

(a) expressing a DNA sequence encoding a mini-proinsulin compound of the formula:

[B(1-30)-Arg-A(1-31)] B(1–30) – Arg – A(1–21)

in a yeast; and

(b) cleaving said mini-proinsulin compound with trypsin.

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